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AMINO ACID COMPOSITION AND PARTIAL SEQUENCE OF XYLANASE FROM AUREOBASIDIUM

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SUMMARY

Although xylanases from five species showed striking similarities in amino acid composition, xylanase from the yeast Aureobasidium, with exceptionally high specific activity, had an atypical composition. Nevertheless, the enzyme possessed extensive identity with a proposed amino-terminal consensus sequence.

INTRODUCTION

Xylanases (endoxylanase, EC 3.2.1.8) occur in numerous genera of bacteria, fungi, algae and protozoa (Dekker and Richards, 1976). In terms of evolution, these microbes were well diverged prior to the appearance of their substrate, xylan, found in the hemicellulose of higher plants (Whistler and Richards, 1970). Multicellular land plants appeared about 415 Myr ago, basidiomycetes and ascomycetes diverged about 700 Myr ago, and the genus Bacillus was established by 750 Myr ago (Walter and Doolittle, 1982; Ochman and Wilson, 1987). Xylanases are thus well-suited for enzyme structure-function studies, as they presumably have relatively recently and independently evolved toward a common function.

A recently described xylanase from Aureobasidium has exceptionally high specific activity (1). This xylanase was purified and analyzed for total amino acid composition and amino-terminal protein sequence. These results are here compared with available literature data on xylanases from diverse sources.

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MATERIALS AND METHODS

Amino acid data and analysis. NRRL strain Y-2311-1, originally identified as a "color variant" of Aureobasidium pullulans (Wickerham and Kurtzman, 1975), was obtained from the ARS Culture Collection, Peoria, Illinois. Since preliminary studies of DNA relatedness have suggested that color variants may be a taxonomically distinct group (Leathers et al, 1988), this strain will be identified in this work simply as Aureobasidium. Xylanase from Aureobasidium was produced and purified to >99% of homogeneity by methods to be reported elsewhere. Total amino acid composition was determined by HPLC of phenylisothiocyanate derivatives. The amino terminus was sequenced by automated Edman degradation. Both analyses were performed at the Genetic Engineering Facility, University of Illinois, Champaign. Amino acid data from xylanases of other organisms were culled from published sources as cited below. Amino-terminal sequences were aligned by inspection. Secondary structure predictions were made by the algorithm of Chou and Fasman (1978), carried out on the Bionet program (IntelliGenetics, Mountain View, California) through the facilities of the Agricultural Research Service Systems Research Resource, Beltsville, Maryland.

RESULTS

Xylanase characteristics. Table 1 summarizes characteristics of xylanases from Aureobasidium (Leathers, 1986), Bacillus subtilis (two isozymes; Bernier, Desrochers et al, 1983; Bernier, Driguez et al, 1983; Paice et al, 1986) Bacillus pumilus (Panbangred et al, 1983; Fukusaki et al, 1984), alkalophilic Bacillus sp. C-125 (xylanase A; Hamamoto et al, 1987), the basidiomycete Schizophyllum commune (xylanase A; Paice et al, 1978), and the ascomycete Talaromyces byssochlamydoides (xylanase X-b-I; Yoskioka et al, 1981). These enzymes encompassed a considerable range of molecular weights, specific activities, and pH and temperature optima. Two thermophilic enzymes were represented, one of which was also described as "alkalophilic," active through pH 10.0. Xylanase from Aureobasidium was exceptional only with regard to specific activity.

TABLE 1. XYLANASE CHARACTERISTICS

SPECIES	MOLECULAR WEIGHT, D	SPECIFIC ACTIVITY, U/mg	pH OPTIMUM	TEMP OPTIMUM, °C
Aureobasidium	20,000	2,100	4.5	45
B. subtilis iso-1	22,000	21	ND[1]	ND
B. subtilis iso-2	32,000	34	5.0	50
B. pumilis	22,384	298	6.5	45-50
B. sp. C-125	42,479	16	6.0-10.0	70
S. commune	31,100	15	5.0	55
T. byssochlamydoides	54,000	117	4.5	70

[1]ND: Not determined.

Amino acid compositions. Total amino acid composition was determined for xylanase purified from Aureobasidium, and compared in Table 2 with literature values for other xylanases from Table 1 (references above). Amino acids were grouped on the basis of chemical

properties (as suggested in Bionet) to reflect conservative differences. In spite of the differences noted in Table 1, striking similarities were found among amino acid group compositions. Apparent extreme values are boxed in Table 2, and were statistically confirmed as follows: Single outliers (a single extreme value within an amino acid group) were the only values to fall above or below the range for the population mean, as predicted by the appropriate t score value at 99% confidence limits. Double outliers (as found in amino acid group B) were confirmed by the $S^2_{n-1,n}/S^2$ method for double outliers (99% confidence limits) as described by Grubbs (1969).

Table 2. XYLANASE AMINO ACID COMPOSITIONS

AMINO ACIDS	SPECIES[1] A	Bs1	Bs2	Bp	Ba	Sc	Tb
Group A: Neutral or Weak Residues, Molar ratio (%)							
Gly	3.93	13.51	18.54	10.53	6.57	15.91	11.76
Ala	11.38	4.86	7.62	6.14	6.31	7.08	7.31
Thr	5.73	12.97	8.89	9.21	3.79	10.04	9.65
Ser	13.24	10.27	11.94	3.95	4.29	12.70	8.37
Pro	1.96	3.24	5.76	2.63	4.04	4.65	4.76
TOTALS	36.24	44.85	52.75	32.46	[25.00]	50.38	41.85
Group B: Small Hydrophilic, Molar ratio (%)							
Asx	12.81	13.51	10.64	11.40	12.63	10.69	12.64
Glx	14.58	3.78	6.15	6.14	13.89	6.44	7.30
TOTALS	[27.39]	17.29	16.79	17.54	[26.52]	17.13	19.94
Group C: Large Hydrophilic, Molar ratio (%)							
His	0	1.08	2.04	2.19	2.53	1.40	1.67
Lys	16.34	2.70	4.95	4.39	4.29	2.41	3.59
Arg	6.61	3.78	3.29	1.75	4.80	1.97	3.36
TOTALS	[22.95]	7.56	10.28	8.33	11.62	5.78	8.62
GROUP D: Small Hydrophobic, Molar ratio (%)							
Val	0	7.57	4.76	4.39	6.06	4.87	5.29
Ile	4.59	3.24	3.15	5.26	4.80	3.95	3.61
Leu	0.62	2.16	3.45	6.58	6.57	3.72	3.55
Met	0	1.08	1.08	3.51	1.26	0.49	1.76
TOTALS	[5.21]	14.05	12.44	19.74	18.69	13.03	14.21
GROUP E: Large Hydrophobic, Molar ratio (%)							
Trp	ND[2]	5.95	ND	2.63	3.03	2.88	4.89
Tyr	0.85	8.11	4.34	7.02	4.55	7.79	3.55
Phe	5.54	2.16	1.09	4.39	3.54	1.68	3.26
TOTALS	>6.39	16.22	>5.43	14.04	11.12	12.35	11.70

1. A: Aureobasidium
   Bs1: Bacillus subtilis isozyme 1
   Bs2: B. subtilis isozyme 2
   Bp: B. pumilis
   Ba: Bacillus sp. C-125
   Sc: Schizophyllum commune
2. ND: Not determined

Xylanase from Aureobasidium showed extreme values for three amino acid groups: Group B (small hydrophilic amino acids), Group C (large hydrophilic amino acids), and Group D (small hydrophobic amino acids).

Group A composition (neutral or weak amino acids) was within the predicted range of the population mean; Group E (large hydrophobic amino acids) could not be fully assessed. With regard to specific amino acids, xylanase from Aureobasidium was atypically high in lysine, and absolutely deficient in histidine, valine, and methionine. Only one other xylanase, that from Bacillus sp. C-125, showed extreme amino acid composition values, in Group A and Group B.

Amino-terminal consensus sequence. Partial amino-terminal sequence was determined for xylanase purified from Aureobasidium. These results are compared in Fig. 1 with literature values for xylanases from Bacillus sp. C-125, B. subtilis (isozyme 1), B. pumilis and S. commune (referenced above). An alignment of these sequences was made (Fig. 1); a similar, although not identical, alignment of sequences from S. commune and Bacillus species was proposed by Paice et al (1986). Xylanase from Aureobasidium showed no apparent sequence relatedness to that from Bacillus sp. C-125.

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Aureobasidium                                                  Ala Gly --- Pro
B. subtilis iso-1                                                          Ala
B. pumilis                          Arg Thr Ile Thr Asn Asn Glu Met Gly Asn His
S. commune                              Ser Gly Thr Pro Ser Ser Thr Gly Thr Asp
CONSENSUS                                      Thr  *   *   *   *  Gly  *   *

                        5                      10                      15
A                  Gly Gly Ile Asp Tyr Val Gln Asn Tyr Asn Gly Asn Leu Gly
Bs                 Ser --- Thr Asp Tyr Trp Gln Asn Trp Thr Asp Gly Gly Gly
Bp                 Ser Gly Tyr Asp Tyr Glu Leu --- Trp Lys Asp Tyr Gly Asn
Sc                 Gly Gly Tyr Tyr Tyr Ser Trp --- Trp Thr Asp Gly Ala Gly
CONSENSUS          Gly Gly Tyr Asp Tyr  *  Gln Asn Trp Thr Asp Gly Gly Gly
                   (Ser)

                                 20                      25                      30
A                  Gln Phe --- Thr Tyr Asn Glu Asn Ala Gly Thr Tyr Ser Met
Bs                 Ile Val Asn Ala Val Asn Gly Ser Gly Gly Asn Tyr Ser Val
Bp                 Thr Ser Met Thr Leu Asn --- Asn Gly Gly Ala Phe Ser Ala
Sc                 Asp Ala --- Thr Tyr
CONSENSUS           *   *   *  Thr Tyr Asn  *  Asn Gly Gly  *  Tyr Ser  *

                              35                      40                      45
A             Tyr Trp Asn Asn Gly Val Asn Gly Asp Phe Val Val Gly Leu Gly
Bs            Asn Trp Ser Asn Thr Gly Asn --- --- Phe Val Val Gly Lys Gly
Bp            Gly Trp Asn Asn Ile Gly Asn Ala Leu Phe Arg Lys Gly Lys Lys
CONSENSUS      *  Trp Asn Asn  *  Gly Asn  *   *  Phe Val Val Gly Lys Gly
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Fig. 1. Amino-terminal xylanase sequences and proposed consensus sequence. Dashes represent spacers inserted into sequences for alignment. Sequences with proposed consensus identity are boxed. Dashed-line boxes enclose amino acids that differ conservatively from the proposed consensus. Asterisks represent breaks in proposed consensus sequence. Numbering is from Aureobasidium xylanase amino terminus.

Secondary structure of xylanase amino terminus. As an initial attempt to interpret the structural significance of the amino-terminal consensus sequence, predictions of secondary structure were made by means of the algorithm of Chou and Fasman (1978). Because this method

averages adjacent residues, a continuous sequence must be used (rather than the consensus of Fig. 1). While all four xylanase sequences shown in Fig. 1 exhibit at least 50% identity with the proposed consensus, that from B. subtilis had the greatest level of identity, at 68%. This sequence was consequently analyzed as a model for the consensus (Fig. 2). An alternating pattern of beta-sheet and beta-turn regions was predicted. Consensus positions appeared to be reasonably poised to stabilize this pattern (Fig. 2). Three beta sheets, separated by turns, may be proposed from these results. A single serine residue in the B. subtilis sequence threatens to disrupt the pattern of the third sheet. As seen in Fig. 1, this residue (corresponding to Aureobasidium xylanase position 33) does not share identity with the proposed consensus.

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Bacillus subtilis isozyme-1: ASTDYWQNWTDGGGIVNAVNGSGGNYSVNWSNTGNFVVGKG
 Chou-Fasman Prediction    :   BBBBBBBBttttBBBBBBttttBBBBBBtBBBBBBttttt
Chou-Fasman Consensus Subset:   BBB BBBBtttt   BBB ttt BB  BtB BBBBtttt

 POTENTIAL STRUCTURE       :   BBBBBBBBttttBBBBBBttttBBBBBBBBBBBBttttt
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Figure 2. Potential secondary structure of xylanase amino terminus. Single-letter amino acid code of amino terminus of xylanase isozyme 1 from B. subtilis and Chou-Fasman predictions from this sequence. B = Beta sheet; t = Beta turn. Consensus subset represents Chou-Fasman predictions at positions included in the proposed consensus sequence.

DISCUSSION

Despite significant differences in enzymes characteriscs, 5 of 7 xylanases compared showed striking similarities in amino acid composition. Four of 5 xylanases showed considerable amino-terminal sequence relatedness; further, enzymes from taxonomically diverse sources showed equivalent, and apparently random, deviations from the consensus. That is, xylanases from Bacillus species were as similar to xylanases from fungi as they were to each other. These results may suggest that xylanases have convergently evolved toward a common structure in accommodation of a common function.

A potential secondary structure consisting of three beta-sheet regions was calculated for the xylanase amino terminus. The potential significance of this prediction, if any, is unknown. An antiparallel pleated beta-sheet region is an important structural feature of lysozyme (Phillips, 1967).

Xylanase from Bacillus sp. C-125 was atypical in amino acid composition, and showed no apparent relatedness to the proposed amino-terminal consensus sequence. This xylanase is atypical in all enzyme characteristics considered in Table 1, and is suited for extreme environments of both high temperature and pH. The novelty of this enzyme in both structure and function might be taken as further evidence in support of independent evolutionary origins of xylanases.

Xylanase from Aureobasidium exhibited extensive amino-terminal consensus identity within 45 residues, representing approximately one-third of the enzyme. However, overall amino acid composition was quite atypical. This suggests that the carboxy-terminal two-thirds of the enzyme may have an unusual structure, possibly one that accounts for the exceptionally high specific activity of the enzyme.

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